REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-01-0188

1. REPORT DATE *(DD-MM-YYYY)*
May 2006

2. REPORT TYPE
Journal Article

3. DATES COVERED *(From - To)*
Aug 2005 - May 2006

4. TITLE AND SUBTITLE
Comparison of Blood Pressure and Thermal Responses in Rats Exposed to Millimeter Wave Energy or Environmental Heat

5a. CONTRACT NUMBER
F41624-01-C-7002

5b. GRANT NUMBER
N/A

5c. PROGRAM ELEMENT NUMBER
62202F

6. AUTHORS
Millenbaugh, N.J., Kiel, J.L., Ryan, K.L., Blystone, R.V., Kalns, J.E., Brott, B.J., Cerna, C.Z., Lawrence, W.S., Soza, L.L., and Mason, P.A.

5d. PROJECT NUMBER
7757

5e. TASK NUMBER
B3

5f. WORK UNIT NUMBER
47

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
General Dynamics Advanced Information Systems
3276 Reliance Loop
Brooks City-Base, TX 78235

8. PERFORMING ORGANIZATION REPORT NUMBER
N/A

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)
Air Force Research Laboratory
Human Effectiveness Directorate
Directed Energy Bioeffects Division, Radio Frequency Radiation Branch
3276 Reliance Loop
Brooks City-Base, Texas 78235

10. SPONSOR/MONITOR'S ACRONYM(S)
AFRL, HEDR

11. SPONSOR/MONITOR'S REPORT NUMBER(S)
AFRL-HE-BR-JA-2005-0021

12. DISTRIBUTION/AVAILABILITY STATEMENT
Approved for public release; distribution unlimited.

13. SUPPLEMENTARY NOTES
AFRL Contract Monitor: Lt Jacque Garcia, (210)-536-2685

14. ABSTRACT
Previous work suggests that sustained exposure to millimeter waves causes greater heating of skin and faster induction of circulatory failure than environmental heat (EH) exposure. We compared temperature changes in skin and the time to reach circulatory collapse in male Sprague-Dawley rats exposed to the following conditions in three separate experiments: (1) EH at 42°C, 35 GHz at 75 mW/cm2, or 94 GHz at 75 mW/cm2 under ketamine and xylazine anesthesia; (2) EH at 43°C, 35 GHz at 90 mW/cm2, or 94 GHz at 90 mW/cm2 under ketamine and xylazine anesthesia; and (3) EH at 42°C, 35 GHz at 90 mW/cm2, or 94 GHz at 75 mW/cm2 under isoflurane anesthesia. In all experiments, temperature increase at the skin surface differed significantly in the rank order of 94 GHz > 35 GHz > EH. Time to reach circulatory collapse was significantly less only for rats exposed to 94 GHz at 90 mW/cm2 compared to both the 35 GHz at 90 mW/cm2 and the EH at 43°C groups. The data indicate that body core heating is the major determinant of induction of hemodynamic collapse in this model of millimeter wave overexposure.

15. SUBJECT TERMS
Radio frequency radiation, microwaves, nonionizing, hyperthermia, stroke, skin

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			Lt Jacque Garcia
U	U	U	UU	8	19B. TELEPHONE NUMBER *(Include area code)* (210) 536-2685

SHOCK, Vol. 25, No. 6, pp. 625–632, 2006

COMPARISON OF BLOOD PRESSURE AND THERMAL RESPONSES IN RATS EXPOSED TO MILLIMETER WAVE ENERGY OR ENVIRONMENTAL HEAT

Nancy J. Millenbaugh,* Johnathan L. Kiel,† Kathy L. Ryan,‡ Robert V. Blystone,§ John E. Kalns,|| Becky J. Brott,§ Cesario Z. Cerna,¶ William S. Lawrence,§ Laura L. Soza,§ and Patrick A. Mason†

**General Dynamics, San Antonio; †Human Effectiveness Directorate, Air Force Research Laboratory, Brooks City-Base; ‡Laboratory Division, US Army Institute of Surgical Research, Fort Sam Houston; §Department of Biology, Trinity University, San Antonio; ||Hyperion Biotechnology, San Antonio; and ¶Conceptual MindWorks, Inc, San Antonio, TX*

Received 19 Oct 2005; first review completed 7 Nov 2005; accepted in final form 22 Dec 2005

ABSTRACT—Electromagnetic fields at millimeter wave lengths are being developed for commercial and military use at power levels that can cause temperature increases in the skin. Previous work suggests that sustained exposure to millimeter waves causes greater heating of skin, leading to faster induction of circulatory failure than exposure to environmental heat (EH). We tested this hypothesis in three separate experiments by comparing temperature changes in skin, subcutis, and colon, and the time to reach circulatory collapse (mean arterial blood pressure, 20 mmHg) in male Sprague-Dawley rats exposed to the following conditions that produced similar rates of body core heating within each experiment: (1) EH at 42°C, 35 GHz at 75 mW/cm^2, or 94 GHz at 75 mW/cm^2 under ketamine and xylazine anesthesia; (2) EH at 43°C, 35 GHz at 90 mW/cm^2, or 94 GHz at 90 mW/cm^2 under ketamine and xylazine anesthesia; and (3) EH at 42°C, 35 GHz at 90 mW/cm^2, or 94 GHz at 75 mW/cm^2 under isoflurane anesthesia. In all three experiments, the rate and amount of temperature increase at the subcutis and skin surface differed significantly in the rank order of 94 GHz more than 35 GHz more than EH. The time to reach circulatory collapse was significantly less only for rats exposed to 94 GHz at 90 mW/cm^2, the group with the greatest rate of skin and subcutis heating of all groups in this study, compared with both the 35 GHz at 90 mW/cm^2 and the EH at 43°C groups. These data indicate that body core heating is the major determinant of induction of hemodynamic collapse, and the influence of heating of the skin and subcutis becomes significant only when a certain threshold rate of heating of these tissues is exceeded.

KEYWORDS—Radio frequency radiation, microwaves, nonionizing, hyperthermia, stroke, skin

ABBREVIATIONS—MMW – millimeter wave, EH – environmental heat, MAP – mean arterial pressure, HR – heart rate, T_C – colonic temperature, T_{SQ} – subcutaneous temperature, T_{surf} – skin surface temperature

INTRODUCTION

Communication, military radar, and weapon detection technologies are being developed that make use of the millimeter wave (MMW) range (frequencies of 3 – 300 GHz) of the electromagnetic spectrum. Some of these emerging technologies involve sources with operating frequencies of 35 and 94 GHz (1) and will use increasingly higher power outputs, which may be capable of causing temperature rises in the skin. As systems are fielded, there will be an increased possibility of brief or prolonged overexposures occurring in maintenance technicians or operators (2). Laboratory and clinical case reports indicate that exposure to radio frequency radiation beyond permissible exposure limits may result in biological effects; however, it is unclear whether these effects result in significant health consequences (2, 3). Thus, there is a continuing interest in studying the potential biological effects of MMW overexposure.

The depth of penetration of electromagnetic fields into an irradiated object decreases as the frequency of the incident field increases (4). Deposition of MMW energy in animals has been calculated to occur within the first 0.78 and 0.32 mm for 30 and 100 GHz, respectively, and thus, is expected to reach only the epidermal and dermal regions of the skin (5). Despite this shallow depth of penetration, it has been shown in rodent models that sustained overexposure to relatively low power densities of 75 mW/cm^2 at frequencies of 35 and 94 GHz can cause significant body core and subcutis heating and changes in heart rate (HR) and mean arterial blood pressure (MAP) (1, 6–10). This heating of internal structures would presumably be caused by thermal conduction.

Physiological mechanisms of MMW-induced cardiovascular changes are not well understood, but the previous researchers noted that some responses such as hypotension with concomitant decreased mesenteric vascular resistance were similar to responses induced by a more conventional method of heat stress, sustained environmental heating (6, 11, 12). Based upon limited available data for MMWs and comparison to results previously reported in the literature for a rat model of environmental heat (EH)–induced shock (6, 11, 13), it was noted that the onset of MMW-induced circulatory collapse

Address reprint requests to Nancy J. Millenbaugh, PhD, Human Effectiveness Directorate, Air Force Research Laboratory, Building 1162, 8262 Hawks Rd, Brooks City-Base, TX 78235. E-mail: nancy.millenbaugh.ctr@brooks.af.mil.

This work was supported by the US Air Force Office of Scientific Research (AFOSR) and Air Force Research Laboratory under US Air Force contract awarded to General Dynamics and by funding from AFOSR to Dr Blystone, Trinity University.
DOI: 10.1097/01.shk.0000209550.11087.fd

occurred at a lower body core temperature (<37.5°C) than EH-induced circulatory collapse (>41.5°C). It was also noted that prolonged exposure to 35 GHz at 75 mW/cm^2 caused a significantly greater rate of heating and temperature increase at the subcutis than at the colon (6). On the basis of these observations, Frei et al. (6) hypothesized that heating in the skin would be greater during MMW than during EH exposures and that a greater stimulation of cutaneous thermoreceptors may explain the variations in the onset of circulatory changes. Data from a subsequent study, however, showed that the onset of the decline in MAP during MMW exposure occurred at colonic temperatures similar to those observed in the previous reports for EH (14). It should be noted that these investigations were not designed to directly compare MMW- and EH-induced thermal and cardiovascular changes and involved variable exposure conditions such as the method of anesthesia.

The current study was designed to test the previously proposed hypotheses by determining if MMW exposure would cause a more rapid induction of hemodynamic collapse than EH exposure within a randomized group of animals. The period of exposure required to elicit circulatory failure, body core temperature at collapse, and temperature changes in the subcutis and at the skin surface were compared during exposure to EH or 35- or 94-GHz MMWs. An anesthetized rat model similar to that used in the previous studies of MMWs- or EH-induced circulatory effects was used. Because it is not possible to accurately monitor the actual dose of energy absorbed by an animal during the experiment, we standardized exposures by using parameters that produced similar rates of increase in body core temperatures for EH and MMW. The type of agent used for anesthesia has been proposed as a factor that could affect MMW-induced shock (14) and other rat models of shock (15–17), and thus, data were collected from rats under isoflurane anesthesia and from rats anesthetized with a combination of ketamine and xylazine. Because the predicted depth of energy deposition is different for 35- and 94-GHz MMWs, both frequencies were studied to determine if the skin heating patterns and blood pressure changes would vary. Also, two power levels for the incident MMW fields, including a power density of 75 mW/cm^2 used in the previous experiments and a higher power level of 90 mW/cm^2, were investigated in this study because data from another study involving radio frequency radiation indicated that the magnitude of cardiovascular responses may be dependent upon incident power density (18).

MATERIALS AND METHODS

Animal care

Fifty-six male Sprague-Dawley rats were obtained from Charles River Laboratories (Raleigh, NC) and were housed individually in standard polycarbonate solid-bottom cages with free access to water. Because the amount of MMW energy absorbed depends upon the structural composition of the skin and the size and shape of the animal (4), rats were weighed twice weekly and maintained on 75% of the diet consumed by rats fed *ad libitum* to maintain animal size and weight throughout the experiments. At the time of experimentation, rats were 3 to 4 months old and weighed between 350 and 400 g (mean ± SD, 382 ± 11 g). A 12:12-h light-dark cycle (lights on at 0600 h) was used, and the room temperature was maintained at 22°C to 24°C. All experimental procedures were conducted between 0800 and 1800 h, with exposures from the different treatment groups randomized throughout each day. The experiments were performed in adherence to the National Institutes of Health Guidelines on the Use of Laboratory Animals and the study was approved by our Institutional Animal Care and Use Committee.

Instrumentation and preparation

Two different methods of anesthesia were used in this study. In the first method, rats received an intraperitoneal injection of a combination of ketamine-HCl (50 mg/kg; Ketaset; Fort Dodge Animal Health, Madison, NJ) and xylazine (10 mg/kg; XYLA-JECT; Phoenix Pharmaceutical, Inc, St. Joseph, Mo) with supplemental doses given as needed during experimentation. Isoflurane was used in a later experiment to confirm results because this agent allowed more consistent maintenance of anesthesia, avoided use of multiple injections during exposures, and was reported to give results most similar to those from unanesthetized rats in standard experimental models of cardiovascular shock (15–17). In this second method, anesthesia was induced using 4% isoflurane and maintained throughout experimentation using 2.5% isoflurane (Isosol; Vedco, St. Joseph, Mo) delivered via a calibrated rodent anesthesia system (IMPAC6; VetEquip, Pleasanton, Calif).

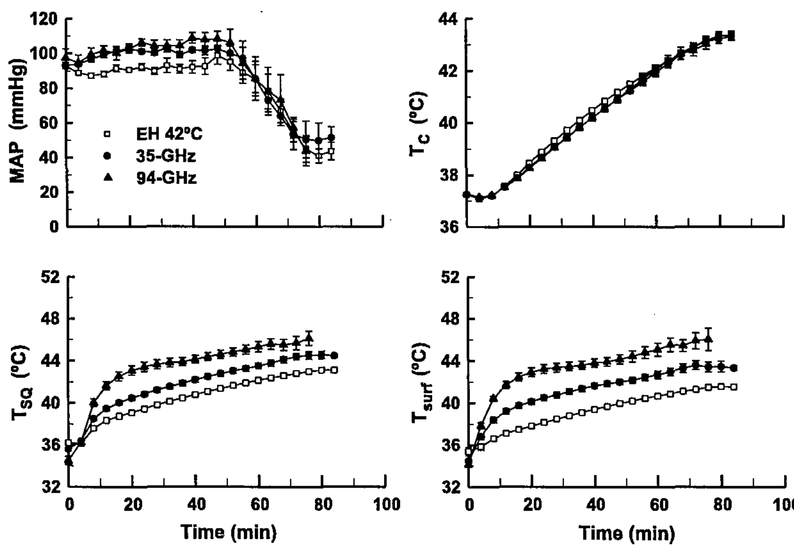

FIG. 1. **MAP and temperature changes during exposure to EH at 42°C or to 35- or 94-GHz MMWs at 75 mW/cm^2 in rats anesthetized with ketamine and xylazine.** Values are mean ± SE. Plots include a 3-min preexposure control period. Time required to reach the end point of MAP of 20 mmHg varied within each exposure group, and mean values are included only up to the time point at which the sample size of each respective group became fewer than 3. T_C indicates colonic temperature; T_{SQ}, left subcutaneous temperature; T_{surf}, left skin surface temperature.

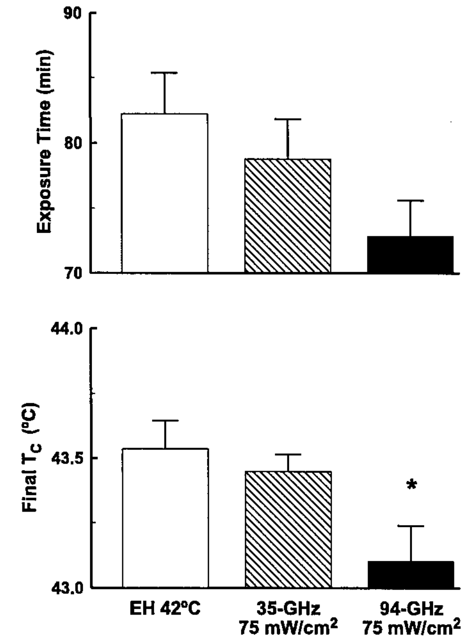

FIG. 2. **Exposure time (time required to reach MAP of 20 mmHg) and final colonic temperature (T_C) in rats anesthetized with ketamine and xylazine and exposed to EH at 42°C, 35 GHz at 75 mW/cm², or 94 GHz at 75 mW/cm².** Values indicate mean ± SE (n = 6 per group). *Significantly different from the value for the EH group ($P < 0.05$).

After induction of anesthesia, the left side was shaved from dorsal midline to ventral midline and from forelimb to hind limb. Colonic temperature (T_C) was maintained at 37.0°C ± 0.5°C using a water-perfused heating pad set at 37.0°C during all surgical procedures. A Teflon catheter (PE-50; DuPont, Wilmington, Del) was surgically placed into the left carotid artery to measure arterial blood pressure. The catheter was attached to a precalibrated blood pressure transducer (Model CP-01; Century, Inglewood, Calif) that was connected to a pressure processor (Model 13-4615-52; Gould Inc, Valley View, Ohio). HR was derived from the arterial pressure signal. Temperatures were monitored at left subcutaneous (lateral, midthoracic, side facing the MMW antenna; T_{SQ}) and colonic (5 – 6 cm postanus) sites using thermistor probes (BSD Medical Corporation, Salt Lake City, Utah). The temperatures from these two sites and the blood pressure and HR data were recorded using a custom-designed acquisition system composed of multichannel interface boxes, analog-to-digital conversion cards, and real-time graphic display using a LabVIEW-based (National Instruments, Austin, Tex) software program.

In addition, left skin surface temperatures (T_{surf}) were measured at a rate of once-per-minute during exposures using an Amber Radiance 1 infrared camera system with ImageDesk software (Raytheon, Goleta, Calif). An external multipoint calibration was performed using a black-body source (Model M340, Mikron Instrument Company, Inc, Oakland, NJ). The skin surface temperatures reported in Figures 1, 3, and 5 were obtained by averaging the temperatures within a 12-pixel diameter circle placed along the same coordinates on each image within a sequence of captured infrared thermograms. The coordinates were selected individually for each rat after exposure and corresponded to the area within the shaved region that gave the highest average temperature.

MMW and EH exposure systems

MMW exposures were conducted in an Eccosorb RF-shielded anechoic chamber at the Radio Frequency Radiation Branch of the Air Force Research Laboratory, Brooks City-Base, Tex. Chamber temperature was maintained at 23.0°C ± 0.5°C during experimentation. Continuous-wave 35- and 94-GHz fields were generated by a Millimeter Wave Exposure System (Applied Electromagnetics, Inc, Atlanta, GA). The generator power output was monitored throughout exposures with a Model 4-32-B Hewlett-Packard power meter. Irradiation was conducted under far-field conditions, with the animal centered along the boresight, at 110 and 80 cm from the antenna for 35 and 94 GHz, respectively. The incident power density of the field was determined at the exposure site with an isotropic probe (Model 8723; Narda Microwave Corporation, Hauppauge, NY) connected to an electromagnetic survey meter (Model 8718; Narda Microwave Corporation). To characterize the distribution of the MMW field power density along the length of the animals, infrared images were obtained of a spatially calibrated carbon-loaded Teflon plate irradiated at the same distance from the horn as the rats. Temperature changes over distance were used to determine that the power density decreased by 50% at 8.5 and 6.7 cm horizontally from the boresight spot on the animal for 35 and 94 GHz, respectively. The percent of body surface area that fell within the region of 50% to 100% of the maximum power density was estimated to be 15.7% ± 1.1% and 12.4% ± 1.0% in 6 pilot animals using body dimensions obtained from spatially calibrated infrared images of the rats and the Meeh equation for calculation of body surface area (19).

For EH exposures, rats were placed inside a custom-made environmental heating chamber composed of a 68 × 44 × 54-cm Styrofoam box (foam insulation; Dow, Midland, Mich) and an in-house developed heat source, which permitted ambient temperature to be controlled with a thermostat (Model 689-0010; Barnant Company, Barrington, Ill). The chamber design allowed heated air to circulate by entering and exiting through vents on one wall. A 7-cm diameter hole was cut in the top of the chamber to accommodate the lens of the infrared camera and allow for T_{surf} acquisition during heating.

Experimental procedure

Three separate experimental protocols were performed, each using rats that were randomly assigned to one of three exposure groups, namely, EH, 35 GHz, or 94 GHz. The three experiments differed either by type of anesthetic or applied power density for MMW and ambient temperature for EH. As mentioned previously, either a mixture of ketamine and xylazine by injection or isoflurane by inhalation was used for anesthesia. Two different incident power densities, 75 or 90 mW/cm², for the MMW exposures and two different ambient temperatures, 42°C or 43°C, for the EH exposures were studied in separate protocols using ketamine- and xylazine-anesthetized rats. These ambient temperature settings for EH were selected so that colonic heating rates for MMW and EH animals would be similar, thus allowing comparison between the three groups within the same experimental protocol. Preliminary experiments indicated that use of either 42°C or 43°C provided colonic heating rates similar to those in animals exposed to MMWs at 75 or 90 mW/cm². In experiment 1, rats were anesthetized with ketamine and xylazine and exposed to EH at 42°C (n = 6), 35 GHz at 75 mW/cm² (n = 6), or 94 GHz at 75 mW/cm² (n = 6). In experiment 2, rats were anesthetized with ketamine and xylazine and exposed to EH at 43°C (n = 6), 35 GHz at 90 mW/cm² (n = 6), or 94 GHz at 90 mW/cm² (n = 6). In experiment 3, rats were anesthetized with isoflurane and exposed to EH at 42°C (n = 6), 35 GHz at 90 mW/cm² (n = 7), or 94 GHz at 75 mW/cm² (n = 7).

After surgery, rats in the EH exposure groups were positioned in the chamber so that the shaved area on the left side of the body faced the top of the chamber. The rat's snout or anesthetic rebreathing tube exited through a 3-cm-diameter port in the side of the chamber, allowing for breathing of room temperature air or anesthetic during uniform whole body heating. All other instrumentation leads and the catheter exited the chamber via a small port. After a 3-min control period (T_C = 37.3°C ± 0.2°C), the heating commenced. At the initiation of heating, approximately 13 min were required to stabilize the target ambient temperature of either 42°C or 43°C; after this stabilization period, ambient temperature was controlled within ± 0.1°C of this value.

After surgery, rats in the MMW exposure groups were placed on a custom-made Styrofoam stand (foam insulation; Dow) positioned either 110 (35 GHz) or 80 cm away (94 GHz) from the antenna horn. The rat was placed in the H orientation (left lateral exposure, long axis of body parallel to magnetic field), with the shaved left side centered in the path of the incident MMW field. The rat was then instrumented with temperature probes and leads for data collection (see Instrumentation and Preparation), and the infrared camera was placed as close to the horn as possible without disturbing the MMW field. After a 3-min control period (T_C = 37.3°C ± 0.2°C), heating via the transmitter commenced at the frequency and power setting specified for the experimental group.

All exposures were continued until the MAP dropped below 20 mmHg. Preliminary experiments showed that this point corresponded to an irreversible decline in MAP with cessation of respiration (unpublished observations), and thus, we defined this as the end point of the study and the duration of EH or MMW exposure was recorded as the exposure time.

Data analysis

Values in the text and figures of the Results section are reported as mean ± SE. The average heating rates for T_C, T_{SQ}, and T_{surf} were calculated as the maximum increase in temperature reached during exposure divided by the exposure time. Statistically significant differences in HR and in the average rates of heating and increases in T_C, T_{SQ}, and T_{surf} were determined using two-way analyses of variance (ANOVAs) applied within each experiment followed by Tukey HSD multiple-comparison test where appropriate. One-way ANOVAs were used for comparisons of exposure times and final T_C's. In all statistical tests, $P < 0.05$ was considered significant.

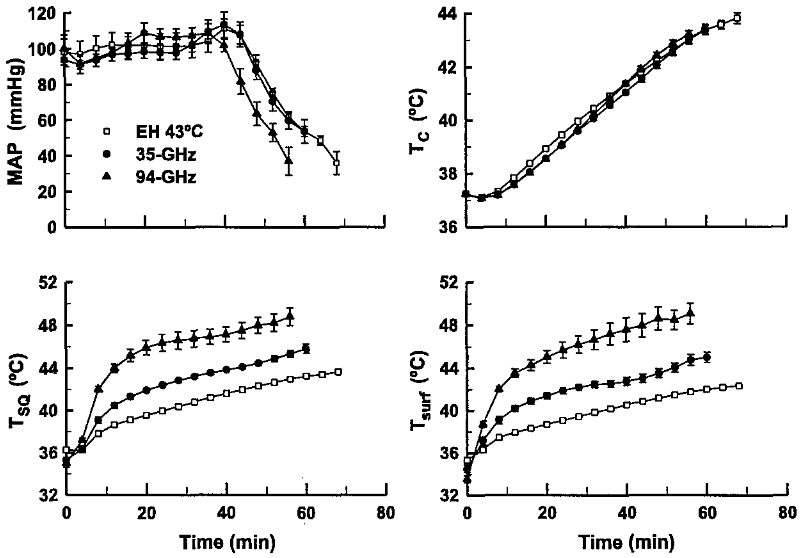

Fig. 3. **MAP and temperature changes during exposure to EH at 43°C, 35 GHz at 90 mW/cm², or 94 GHz at 90 mW/cm² in rats anesthetized with ketamine and xylazine.** Values are mean ± SE. Plots include a 3-min preexposure control period. Time required to reach the end point of MAP of 20 mmHg varied within each exposure group, and mean values are included only up to the time point at which the sample size of each respective group became fewer than 3. T_C indicates colonic temperature; T_{SQ}, left subcutaneous temperature; T_{surf}, left skin surface temperature.

RESULTS

Experiment 1: Ketamine- and xylazine-anesthetized rats exposed to EH at 42°C or MMWs at 75 mW/cm²

Changes in MAP, T_C, T_{SQ}, and T_{surf} in rats anesthetized with ketamine and xylazine and exposed to a 3-min control period followed by EH at 42°C or 35 or 94 GHz at 75 mW/cm² are shown in Figure 1. The duration of exposure required to reach the end point of MAP of 20 mmHg varied for individual animals within each group. Mean values in Figure 1 are included only up to the time point at which the sample size of each respective group became fewer than three, that is, four or more animals had reached the MAP end point and were no longer receiving the exposure. MAP, HR, body core temperatures, and peripheral temperatures exhibited a pattern of changes typical of those previously observed during EH and 35- and 94-GHz exposures (1, 6, 12). In all three exposure groups, MAP remained relatively constant or increased slightly until a point at which it began to decline rapidly until death. HR increased over time in all groups as expected, but no significant differences between the groups were detected (data not shown). The heating profiles for T_{SQ} and T_{surf} for all groups exhibited two distinct phases of heating, an early phase of rapid temperature increase, and a second phase with slower temperature increase. In the MMW groups, T_{SQ} and T_{surf} began to increase immediately upon initiation of exposure, whereas a lag time occurred for increases in T_C. This pattern was also observed in the EH group, although the initial increases in T_{SQ} and T_{surf} were not as great as in the MMW groups.

Changes in T_C, T_{SQ}, and T_{surf} were compared using two-way ANOVAs (Table 1). An interaction effect was detected between the two factors, exposure group and site of temperature measurement ($P < 0.0001$). Within-group comparisons of T_C, T_{SQ}, and T_{surf} showed that increases and average rates of increase for T_{SQ} and T_{surf} were significantly greater than the increases and rates of heating for T_C in both MMW exposure groups (all $P < 0.001$). In contrast, no differences were detected for total and average rate of increase in T_C, T_{SQ}, and T_{surf} in the EH group.

Because exposure settings were selected so that the rates of temperature rise in body core for all three treatment groups would be similar, the average heating rate for T_C did not differ among groups. In addition, the increases in T_C did not differ among the three exposure groups. In contrast to heating at the body core, the average heating rate and the increase in T_{SQ} and T_{surf} were greater in both MMW groups compared with the EH group and were greater for 94 GHz compared with 35 GHz.

This trend of decreasing rate and extent of heating at subcutis and skin surface in the rank order of 94 GHz > 35 GHz > EH groups (Table 1) corresponded to a general pattern of increasing exposure times and final T_C's (Fig. 2). The average exposure times from initiation of exposure to circulatory collapse in the 94-GHz, 35-GHz, and EH groups were 72.8 ± 2.8, 78.8 ± 3.1, and 82.2 ± 3.1 min, respectively. However, despite this increasing trend in the values, the differences did not reach statistical significance. The final T_C in the 94-GHz group was 43.1°C ± 0.1°C and was statistically different from but only slightly lower than the final T_C of 43.5°C ± 0.1°C in the EH group ($P = 0.03$).

Experiment 2: Ketamine- and xylazine-anesthetized rats exposed to EH at 43°C or MMWs at 90 mW/cm²

Rats in experiment 2 were exposed to a higher ambient temperature of 43°C in the EH group and a higher incident power density of 90 mW/cm² in the 35- and 94-GHz MMW groups. Figure 3 shows that the time course of changes in MAP, T_C, T_{SQ}, and T_{surf} for the three exposure groups were similar to those observed in experiment 1. Also, as observed in experiment 1, HR increased over time in all groups with no

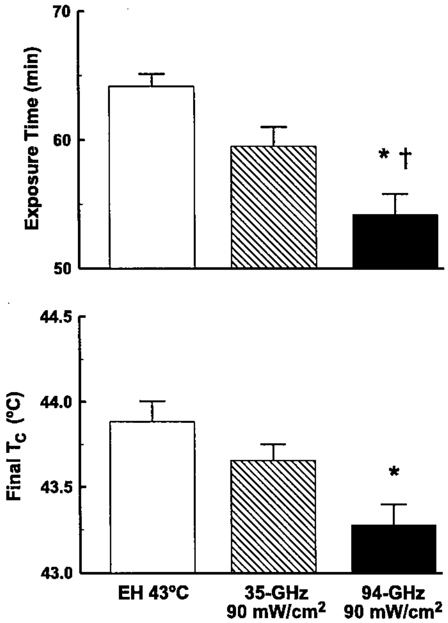

FIG. 4. **Exposure time and final colonic temperature (T_C) in rats anesthetized with ketamine and xylazine and exposed to EH at 43°C, 35 GHz at 90 mW/cm^2 or 94 GHz at 90 mW/cm^2.** Values indicate mean ± SE (n = 6 per group). *Significantly different from the value for the EH group (P < 0.05). †Significantly different from value for the 35-GHz group (P < 0.05).

detectable between-group differences (data not shown). The increase and average rates of increase for T_C did not differ among the three exposure groups (Table 1). In addition, the average heating rate and the increase in T_{SQ} and T_{surf} were greater in both MMW groups compared with that of the EH group and were greater for 94 GHz than 35 GHz. Within-group comparisons of temperature increases and heating rates for T_C, T_{SQ}, and T_{surf} yielded similar results as in experiment 1. In the MMW exposure groups, increases and average rates of heating for T_{SQ} and T_{surf} were significantly greater than increases and rates of heating for T_C (all P < 0.001). No differences were detected for total and average rates of increase for T_C, T_{SQ}, and T_{surf} in the EH group.

As in experiment 1, a general trend of increasing exposure times and final T_C's was observed (Fig. 4), which corresponded to a trend of decreasing rate and extent of heating of subcutis and skin surface in the order of 94 GHz > 35 GHz > EH (Table 1). The exposure time in rats exposed to 94 GHz was 54.2 ± 1.6 min and was significantly shorter than exposure times in the EH and 35-GHz groups, which were 64.2 ± 1.0 and 59.5 ± 1.5 min, respectively (Fig. 4). In addition, the final T_C in the 94-GHz group was significantly lower by 0.6°C compared with the final T_C in the EH group (P = 0.006).

Experiment 3: Isoflurane-anesthetized rats exposed to EH at 42°C, 35 GHz at 90 mW/cm^2, or 94 GHz at 75 mW/cm^2

We performed the same protocol as in experiment 1 with isoflurane to determine if using a different anesthetic would provide similar results. Figure 5 shows changes in MAP, T_C, T_{SQ}, and T_{surf} in rats anesthetized with isoflurane and exposed to EH at 42°C, 35 GHz at 90 mW/cm^2, or 94 GHz at 75 mW/cm^2. Qualitatively, the patterns of changes are similar to those observed in rats anesthetized with ketamine and xylazine. HR increased over time in all groups, but no between-group differences were detected (data not shown). The increases in T_C and average rates of heating for T_C were the same in all three exposure groups (Table 1). The rate of increase and amount of increase in T_{SQ} and T_{surf} were greater in both MMW groups than in the EH group and were greater for the 94 versus the 35-GHz group. Within-group comparisons of changes in T_C, T_{SQ}, and T_{surf} showed that heating was greater at both of the peripheral sites (all P's < 0.001) than at the body core for the 35- and 94-GHz groups. In the rats exposed to EH, no differences were detected for the changes in T_C, T_{SQ}, and T_{surf}. Exposure times and final T_C's did not differ significantly among the three exposure groups (Fig. 6).

DISCUSSION

Blood pressure and thermal changes in anesthetized rats in response to prolonged EH or 35- or 94-GHz MMW exposures have been previously documented (1, 6–14, 20–23). These methods of inducing thermal stress were, however, investigated in separate studies, and direct statistical comparison of data was not possible. The purpose of the current study was to compare changes in MAP and colonic, subcutaneous, and skin surface temperatures during sustained exposure to EH or to 35- or 94-GHz irradiation within a randomized group of subjects.

The mechanisms involved in elicitation of cardiovascular responses by MMW exposure are not well understood, although it is generally held that responses to radio frequency radiation overexposure are mainly due to heating (3). Because thermoreceptors are located peripherally and in deeper body regions (24), it is possible that activation of the receptors at both sites could affect the cardiovascular system. Temperatures were therefore monitored at the body core and at the subcutis and skin surface. Colonic temperature was used as an indicator of heat stress at central sensory receptors because it can be monitored noninvasively and avoids possible disruption of the normal thermoregulatory mechanisms by placing a probe in the hypothalamus.

Differences in the experimental procedures used for exposure to EH and MMW are a possible source of variation in the actual dose of energy delivered to the animals in different groups within each experiment. The MMW-generating device only allows one side of the animal to directly face the incident MMW field and, as mentioned previously in Materials and Methods, it was estimated that only 15.7% ± 1.1% and 12.4% ± 1.0% of a rat's body surface would receive 50% to 100% of the maximum dose of 35- or 94-GHz MMWs, respectively. In contrast, the animal's body except for the nose was placed into the warm air chamber for the EH exposures. Because it was not possible to accurately measure the dose of energy absorbed by the animals during the exposures to EH or MMWs, the colonic heating rates were matched for EH and MMW groups within each experimental series. This provided a means to standardize exposures by subjecting animals in different

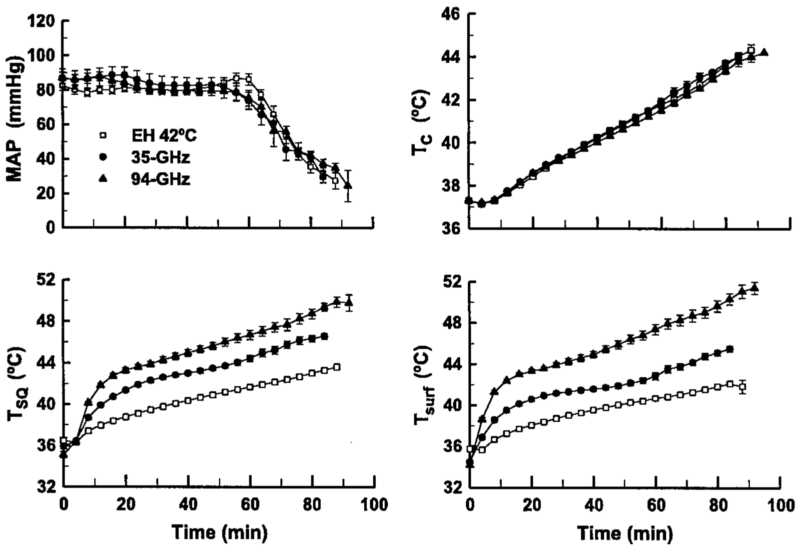

FIG. 5. **MAP and temperature changes during exposure to EH at 42°C, 35 GHz at 90 mW/cm², or 94 GHz at 75 mW/cm² in rats anesthetized with isoflurane.** Values are mean ± SE. Plots include a 3-min preexposure control period. Time required to reach the end point of MAP of 20 mmHg varied within each exposure group, and mean values are included only up to the time point at which the sample size of each respective group became fewer than 3. T_C indicates colonic temperature; T_{SQ}, left subcutaneous temperature; T_{surf}, left skin surface temperature.

exposure groups to similar levels of thermal stress at the level of deep body thermoreceptors.

Based upon data from rats exposed to 35 GHz at 75 mW/cm², Jauchem and Frei (25) and Frei et al. (6) proposed that MMW exposures produce greater temperature differentials between the skin and colon than would be expected during EH exposures. Indeed, in all three experiments in the current investigation, we consistently observed that MMW exposures produced larger and more rapid increases in T_{SQ} and T_{surf} than in EH exposures. Because colonic heating rates did not differ between MMW and EH groups within the same experiment, this resulted in thermal gradients between the central and superficial body regions during EH exposures that were of lesser magnitude than the differences observed in the MMW groups. Thus, the animal model in this study exhibited similar body core heating but significantly different levels of heating at the subcutis and skin surface tissues and possibly different levels of stimulation of peripheral thermoreceptors for rats in the EH and 35- and 94-GHz exposure groups.

Frei et al. (6), Ryan et al. (7), and Jauchem et al. (8) hypothesized that prolonged 35-GHz exposure could cause hemodynamic collapse in a shorter time frame and at a lower core temperature than EH exposure due to the higher skin temperatures reached during the MMW exposures. It was suggested that the faster heating and higher temperatures reached in skin influences the magnitude of the circulatory response. Indeed, in both experiments involving use of ketamine and xylazine in the current study, exposure times and final T_C's declined in the rank order of EH >35 GHz >94 GHz as rise in temperature, average heating rate, and final temperature increased at skin surface and subcutis. However, differences in final T_C only reached significance for the EH group compared with the 94-GHz group under ketamine and xylazine anesthesia (experiments 1 and 2), and this difference was not detected in rats anesthetized with isoflurane (experiment 3). Furthermore, differences in exposure time only reached significance in experiment 2 for rats exposed to 94 GHz at 90 mW/cm² compared with rats exposed to EH at 43°C or 35 GHz at 90 mW/cm². Rats exposed to 94 GHz at 90 mW/cm² exhibited the greatest rate of heating for T_{SQ} and T_{surf} of all groups in this study. These data support the hypothesis that greater heating of superficial tissues by MMWs, in addition to the heating at the body core, has an effect on induction of circulatory failure. In addition, because of the noted trends mentioned above, these results indicate that this phenomenon becomes significant only when a certain rate of heating of subcutis and skin surface is exceeded.

The amount of the incident radio frequency radiation energy absorbed by an organism and the depth of penetration into the organism depends upon several factors, including the frequency of the field and the size and shape of the organism (4). Resultantly, the amount of energy deposited and, thus, the heating profile as a function of distance through the skin tissue may vary with frequency. No validated mathematical models are currently available for accurate prediction of the amount of MMW energy absorbed, the amount of energy deposited at specific layers within the skin, or the transfer of heat throughout all the tissues during prolonged exposures of the rat. It has been estimated, however, that 51% and 68% of the incident MMW power is transmitted into a planar section of skin for 30 and 100 GHz, respectively (5). Indeed, the data from the current study show that some responses in the rat were significantly different for 35 GHz compared with 94 GHz, in agreement with this estimation. Temperature increases at the subcutis and skin surface were greater for 94 GHz in all three experiments, and exposure time was significantly less for 94 GHz in experiment 2. Therefore, the frequency-specific differences in heating of the subcutis and skin surface and in the circulatory

TABLE 1. **Temperature increases and heating rates at monitored sites in rats anesthetized with Ketamine and Xylazine (experiments 1 and 2) or Isoflurane (experiment 3)**

	Colonic (T_C)	Left subcutaneous (T_{SQ})	Left skin surface (T_{surf})
Experiment 1			
Total increase in temperature (°C)			
EH at 42°C (n = 6)	6.4 ± 0.1	7.2 ± 0.1	6.3 ± 0.3
35 GHz at 75 mW/cm² (n = 6)	6.3 ± 0.1	9.5 ± 0.2*	9.6 ± 0.1*
94 GHz at 75 mW/cm² (n = 6)	5.9 ± 0.1	12.1 ± 0.4*†	12.4 ± 0.7*†
Average heating rate (°C/min)			
EH at 42°C	0.078 ± 0.003	0.088 ± 0.003	0.076 ± 0.003
35 GHz at 75 mW/cm²	0.081 ± 0.003	0.121 ± 0.005*	0.122 ± 0.004*
94 GHz at 75 mW/cm²	0.082 ± 0.003	0.167 ± 0.009*†	0.172 ± 0.014*†
Experiment 2			
Total increase in temperature (°C)			
EH at 43°C (n = 6)	6.8 ± 0.1	7.4 ± 0.2	7.1 ± 0.3
35 GHz at 90 mW/cm² (n = 6)	6.5 ± 0.1	11.0 ± 0.4*	11.1 ± 0.8*
94 GHz at 90 mW/cm² (n = 6)	6.2 ± 0.1	14.6 ± 0.7*†	16.2 ± 0.9*†
Average heating rate (°C/min)			
EH at 43°C	0.105 ± 0.002	0.116 ± 0.002	0.110 ± 0.007
35 GHz at 90 mW/cm²	0.110 ± 0.003	0.185 ± 0.006*	0.186 ± 0.013*
94 GHz at 90 mW/cm²	0.114 ± 0.002	0.271 ± 0.018*†	0.302 ± 0.022*†
Experiment 3			
Total increase in temperature (°C)			
EH at 42°C (n = 6)	7.1 ± 0.2	7.4 ± 0.3	6.6 ± 0.2
35 GHz at 90 mW/cm² (n = 7)	6.8 ± 0.2	11.1 ± 0.3*	11.3 ± 0.4*‡
94 GHz at 75 mW/cm² (n = 7)	6.3 ± 0.3	14.5 ± 0.4*†	16.1 ± 0.5*†
Average heating rate (°C/min)			
EH at 42°C	0.084 ± 0.002	0.087 ± 0.004	0.079 ± 0.003
35 GHz at 90 mW/cm²	0.088 ± 0.003	0.145 ± 0.009*	0.149 ± 0.006*‡
94 GHz at 75 mW/cm²	0.081 ± 0.002	0.185 ± 0.008*†	0.206 ± 0.009*†

Values are means ± SE.
$P < 0.0001$ in all three experiments for interaction effect detected by two-way ANOVA.
*Significantly different from value in EH group ($P < 0.05$).
†Significantly different from value in 35-GHz group ($P < 0.05$).
‡n = 6 for T_{surf} measurements in this group.

responses observed in this study may be explained at least in part by differences in the amounts of absorbed energy.

Because of higher temperatures reached in skin during MMW heating, it is possible that thermal injury of skin or release of humoral mediators from skin, in addition to cutaneous thermoreceptor stimulation, during MMW exposures may be involved in the observed phenomenon of shortened time course of induction of hemodynamic collapse. This possibility was discussed previously by Frei et al. (6), who noted that cardiovascular changes evoked by exposure to 35 GHz at 75 mW/cm^2 were quite different from those observed in well-established burn models, and that the time course of cardiovascular changes does not coincide with previous reports of the more delayed timing of the release of known humoral substances with vasoactivity. Also, to date, no known humoral mediators of MMW effects that correspond to the time course of onset of MAP decrease in this study have been reported for *in vivo* models. In fact, results of previous investigations suggest that common mediators involved in several forms of shock such as nitric oxide, platelet activating factor, and histamine do not play a role in MMW-induced circulatory collapse (7, 20–23).

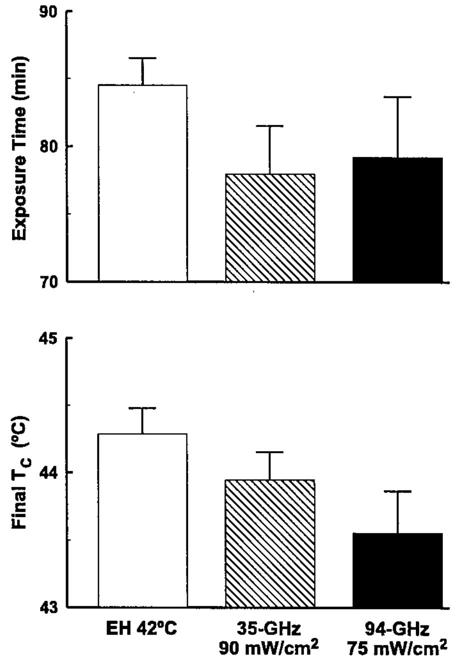

FIG. 6. **Exposure time and final colonic temperature (T_C) in rats anesthetized with isoflurane and exposed to EH at 42°C (n = 6), 35 GHz at 90 mW/cm² (n = 7), or 94 GHz at 75 mW/cm² (n = 7).** Values are mean ± SE.

Different anesthetic regimens have been used in the previous investigations of thermal and cardiovascular responses in rats to sustained EH or MMW heating (1, 6–9, 12, 14). Using ketamine or pentobarbital, Frei et al. (6) and Ryan et al. (7, 10, 21) observed a decrease in MAP at a T_C less than 40°C and noted that this was lower than that previously reported for EH (T_C > 41.5°C) (11). However, Kalns et al. (14) used urethane anesthesia and observed that MAP started to decrease during 35-GHz exposure for $T_C \geq 41.5$°C, indicating that this response may be dependent upon the specific agent used for anesthesia. Anesthetics are known to affect central and peripheral thermoregulatory mechanisms in rats, and individual agents have been shown to have varying degrees of influence on control of body temperature (26–28).

In the current study, skin temperatures during prolonged MMW heating reached a maximum of 52.5°C. This temperature exceeds the reported human thermal pain threshold of 43.9°C for 94-GHz MMWs (29) and meets or exceeds temperatures of 48°C to 52°C known to elicit pain behaviors in unanesthetized Sprague-Dawley rats in response to other types of thermal stimuli such as a hot plate or water bath (30, 31). Therefore, it was unethical to expose unanesthetized animals to prolonged MMW heating, and we obtained results using both isoflurane and a mixture of ketamine and xylazine. Although direct statistical comparisons of results from ketamine and xylazine versus isoflurane anesthetized rats in experiments 1 and 3 could not be performed, some qualitative similarities and differences can be noted. General patterns of changes in MAP, T_C, T_{SQ}, and T_{surf} were similar, and exposure times did not differ among exposure groups within the individual experiments. However, final T_C was significantly less (P = 0.03) for the ketamine and xylazine rats exposed to 94 GHz at 75 mW/cm^2 compared with the EH-exposed rats but did not differ in the isoflurane-anesthetized rats. The detected difference in the ketamine and xylazine groups was only 0.4°C and, although statistically significant, is not expected to have major physiological relevance.

In summary, the current data show that MMW exposure caused greater temperature differentials between body core and peripheral tissues than EH exposure, and exposure to 94 GHz caused the greatest differential between these two body sites. A pattern of decreasing exposure time and final T_C was observed for MMW versus EH exposures, but these differences reached statistical significance only in the rats exposed to 94-GHz MMW at a power density of 90 mW/cm^2 compared with EH at 43°C using ketamine and xylazine. The results indicate that body core heating is the major determinant of induction of circulatory failure, and that the influence of heating of the skin and subcutis becomes significant only when a certain threshold rate of heating of these tissues is exceeded. Overall, the data suggest that MMWs induce the same thermoregulatory responses as a warm ambient environment, and that differences in induction of circulatory failure for MMWs versus warm air can be explained by observed variations in temperature changes. This is in agreement with recent reviews (2, 3) that have discussed the role of thermal effects in the potential health consequences of overexposure to MMWs.

ACKNOWLEDGMENTS

All experiments and animal care procedures were approved by the Institutional Animal Care and Use Committee of the Air Force Research Laboratory, Brooks City-Base, Tex, and were conducted in compliance with the *Guide for the Care and Use of Laboratory Animals* prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources–National Research Council and US laws.

REFERENCES

1. Jauchem JR, Ryan KL, Frei MR: Cardiovascular and thermal responses in rats during 94 GHz irradiation. *Bioelectromagnetics* 20:264–267, 1999.
2. Ryan KL, D'Andrea JA, Jauchem JR, Mason PA: Radio frequency radiation of millimeter wave length: potential occupational safety issues relating to surface heating. *Health Phys* 78:170–181, 2000.
3. Medical aspects of radio frequency radiation overexposure. *Health Phys* 82: 387–391, 2002.
4. Durney C, Massoudi H, Iskander M: *Radiofrequency Radiation Dosimetry Handbook*, 4th ed. Brooks Air Force Base, Texas: US Air Force School of Aerospace Medicine, 1986. Technical Report USAFSAM 85-73.
5. Gandhi OP, Riazi A: Absorption of millimeter waves by human beings and its biological implications. *IEEE Trans Microwave Theor Tech* 34: 228–236, 1986.
6. Frei MR, Ryan KL, Berger RE, Jauchem JR: Sustained 35-GHz radio frequency irradiation induces circulatory failure. *Shock* 4:289–293, 1995.
7. Ryan KL, Frei MR, Berger RE, Jauchem JR: Does nitric oxide mediate circulatory failure induced by 35 GHz microwave heating? *Shock* 6: 71–76, 1996.
8. Jauchem JR, Ryan KL, Lovelace JD, Frei MR: Effects of esmolol on 35 GHz microwave-induced lethal heat stress. *J Auton Pharmacol* 17:165–174, 1997.
9. Ryan KL, Walters TJ, Tehrany MR, Lovelace JD, Jauchem JR: Age does not affect thermal and cardiorespiratory responses to microwave heating in calorically restricted rats. *Shock* 8:55–60, 1997.
10. Ryan KL, Lovelace JD, Frei MR, Jauchem JR: Administration of a nitric oxide donor does not affect hypotension induced by 35-GHz microwave heating. *Methods Find Exp Clin Pharmacol* 19:455–464, 1997.
11. Kregel KC, Wall PT, Gisolfi CV: Peripheral vascular responses to hyperthermia in the rat. *J Appl Physiol* 64: 2582–2588, 1988.
12. Ryan KL, Tehrany MR, Jauchem JR: Nitric oxide does not contribute to the hypotension of heatstroke. *J Appl Physiol* 90:961–970, 2001.
13. Hubbard RW, Bowers WD, Matthew WT, Curtis FC, Criss RE, Sheldon GM, Ratteree JW: Rat model of acute heatstroke mortality. *J Appl Physiol* 42: 809–816, 1977.
14. Kalns J, Ryan KL, Mason PA, Bruno JG, Gooden R, Kiel JL: Oxidative stress precedes circulatory failure induced by 35-GHz microwave heating. *Shock* 13: 52–59, 2000.
15. Debaene B, Goldfarb G, Braillon A, Jolis P, Lebrec D: Effects of ketamine, halothane, enflurane, and isoflurane on systemic and splanchnic hemodynamics in normovolemic and hypovolemic cirrhotic rats. *Anesthesiology* 73:118–124, 1990.
16. Schaefer CF, Biber B, Brackett DJ, Schmidt CC, Fagraeus L, Wilson MF: Choice of anesthetic alters the circulatory shock pattern as gauged by conscious rat endotoxemia. *Acta Anaesthesiol Scand* 31:550–556, 1987.
17. Seyde WC, Longnecker DE: Anesthetic influences on regional hemodynamics in normal and hemorrhaged rats. *Anesthesiology* 61:686–698, 1984.
18. Jauchem JR, Frei MR, Heinmets F: Heart rate changes due to 5.6-GHz radiofrequency radiation: relation to average power density. *Proc Soc Exp Biol Med* 177:383–387, 1984.
19. Gilpin DA: Calculation of a new Meeh constant and experimental determination of burn size. *Burns* 22:607–611, 1996.
20. Jauchem JR, Ryan KL, Tehrany MR: Effects of histamine receptor blockade on cardiovascular changes induced by 35 GHz radio frequency radiation heating. *Auton Autacoid Pharmacol* 24:17–28, 2004.
21. Ryan KL, Frei MR, Jauchem JR: Circulatory failure induced by 35 GHz microwave heating: effects of chronic nitric oxide synthesis inhibition. *Shock* 7:70–76, 1997.
22. Ryan KL, Jauchem JR, Tehrany MR, Boyle HL: Platelet-activating factor does not mediate circulatory failure induced by 35-GHz microwave heating. *Methods Find Exp Clin Pharmacol* 24:279–286, 2002.
23. Ryan KL, Lovelace JD, Frei MR, Jauchem JR: Administration of a nitric oxide donor does not affect hypotension induced by 35-GHz microwave heating. *Methods Find Exp Clin Pharmacol* 19:455–464, 1997.
24. Gordon CJ: *Temperature Regulation in Laboratory Rodents*. New York: Cambridge University Press pp 19–46, 1993.
25. Jauchem JR, Frei MR: Heart rate and blood pressure changes during radiofrequency irradiation and environmental heating. *Comp Biochem Physiol* 101A:1–9, 1992.
26. Wixson SK, White WJ, Hughes HC, Lang CM, Marshall WK: The effects of pentobarbital, fentanyl-droperidol, ketamine-xylazine and ketamine-diazepam on core and surface body temperature in adult male rats. *Lab Anim Sci* 37: 743–749, 1987.
27. Refinetti R, Carlisle HJ: Thermoregulation during pentobarbital and ketamine anesthesia in rats. *J Physiol (Paris)* 83:300–303, 1988–1989.
28. Kushikata T, Hirota K, Kotani N, Yoshida H, Kudo M, Matsuki A: Isoflurane increases norepinephrine release in the rat preoptic area and the posterior hypothalamus in vivo and in vitro: relevance to thermoregulation during anesthesia. *Neuroscience* 131:79–86, 2005.
29. Walters TJ, Blick DW, Johnson LR, Adair ER, Foster KR: Heating and pain sensation produced in human skin by millimeter waves: comparison to a simple thermal model. *Health Phys* 78:259–267, 2000.
30. Baliki M, Calvo O, Chialvo DR, Apkarian AV: Spared nerve injury rats exhibit thermal hyperalgesia on an automated operant dynamic thermal escape task. *Mol Pain* 1:18, 2005.
31. Williams DG, Dickenson A, Fitzgerald M, Howard RF: Developmental regulation of codeine analgesia in the rat. *Anesthesiology* 100:92–97, 2004.